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## Effect of pramlintide on satiety and food intake in obese subjects and subjects with type 2 diabetes

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**Abstract** *Aims/hypothesis:* Long-term trials in insulin-treated subjects with type 2 diabetes have shown that adjunctive treatment with the amylin analogue pramlintide reduces HbA<sub>1c</sub> levels and elicits weight loss. While amylin reduces food intake in rodents, pramlintide's effect on satiety and food intake in humans has not yet been assessed. *Methods:* In this randomised, double-blind, placebo-controlled crossover study, 11 insulin-treated men with type 2 diabetes (age 60±9 years, BMI 28.9±4.8 kg/m<sup>2</sup>) and 15 non-diabetic obese men (age 41±21 years, BMI 34.4±4.5 kg/m<sup>2</sup>) underwent two standardised meal tests. After fasting overnight, subjects received single subcutaneous injections of either pramlintide (120 µg) or placebo, followed by a pre-load meal. After 1 h, subjects ate an ad libitum buffet meal. Energy intake and meal duration were measured, as were hunger ratings (using visual analogue scales), and plasma cholecystokinin, glucagon-like peptide-1 and peptide YY concentrations over time. *Results:* Compared with placebo, pramlintide reduced energy intake in both the type 2 diabetes ( $\Delta$ -202±64 kcal, -23±8%,  $p$ <0.01) and obese ( $\Delta$ -170±68 kcal, -16±6%,  $p$ <0.02) groups, without affecting meal duration. Hunger and hormonal analyte profiles provided evidence that pramlintide may exert a primary satiogenic effect, independently of other anorexigenic gut

peptides. *Conclusions/interpretation:* The results indicate that enhanced satiety and reduced food intake may explain the weight loss observed in long-term pramlintide trials.

**Keywords** Amylin · Hunger · Obesity · Peptide hormones · Satiety

**Abbreviations** CNS: central nervous system · CCK: cholecystokinin · GLP-1: glucagon-like peptide-1 · ITT: intent-to-treat · LS: least squares · PYY: peptide YY · VAS: visual analogue scale

### Introduction

In healthy subjects, food intake triggers the release of numerous pancreatic and gastrointestinal peptide hormones that play an important role in the regulation of postprandial metabolism. This regulation may include the quantity of food consumed, as well as the absorption and disposition of ingested nutrients [1–5]. Specifically, there is growing recognition that peptide hormones secreted from the gut and from pancreatic islets in response to meals may act as humoral and/or neuronal feedback signals to the central nervous system (CNS), mediating the perception of fullness and satiation during meals (thus contributing to meal termination), and/or the maintenance of satiety after meals (thus affecting inter-meal intervals) [1–5]. Whether a given peptide hormone has a physiological role in the regulation of food intake and body weight is typically established by supporting evidence from animal studies (using gain-of-function studies with the peptide itself and loss-of-function studies with selective antagonists and/or gene knock-outs) and from clinical studies assessing the effect of the peptide on satiety and food intake in humans [1–5]. To date, randomised, controlled crossover studies in humans have reported an increase in food intake at a buffet meal following the administration of the gastric hormone ghrelin [6], and a decrease following the administration of the gut hormones cholecystokinin (CCK) [7, 8], glucagon-like peptide-1 (GLP-1) [9–11], oxyntomodulin [12] and peptide YY3–36

Duality of interest. C. Weyer, Y. Wang, C. Burns, C. Lush and S. Strobel are employees of and shareholders in Amylin Pharmaceuticals (San Diego, CA, USA). I. Chapman, B. Parker, S. Doran, C. Feinle-Bisset, J. Wishart and M. Horowitz are employees of the Royal Adelaide Hospital.

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[13, 14], and the islet hormones glucagon [15], pancreatic polypeptide [16] and somatostatin [17].

Amylin is a 37-amino-acid peptide hormone that is normally co-secreted with insulin from pancreatic beta cells in response to meals [18]. Amylin acts as a neuroendocrine hormone that binds with high affinity to specific amylin receptors in the CNS, most notably the nucleus accumbens, dorsal raphe and area postrema [19–21], and exerts various glucoregulatory and anorexigenic effects [18]. Amylin complements the effects of insulin in postprandial glucose homeostasis by regulating the rate of glucose appearance in the circulation via suppression of nutrient-stimulated glucagon secretion and slowing of gastric emptying [18]. In addition, numerous non-clinical studies have provided evidence that amylin is involved in the central regulation of food intake and body weight [22–27]. Specifically, central or peripheral administration of amylin was shown to reduce food intake and body weight in rodents in a dose-dependent manner [22–25], whereas central or peripheral administration of a selective amylin antagonist increased food intake and body fat stores [26, 27].

Pramlintide, a soluble synthetic analogue of human amylin, was recently approved in the United States to be given at mealtimes as an adjunct to mealtime insulin therapy in patients with type 1 or type 2 diabetes who have failed to achieve desired glucose control, despite optimal insulin therapy with or without a concurrent sulphonylurea agent and/or metformin respectively [28, 29]. Also, pramlintide is currently in clinical development as a potential treatment for obesity in individuals without diabetes. Consistent with the observed glucoregulatory actions of amylin in rodents, clinical studies in insulin-treated patients with diabetes have shown that pramlintide reduces postprandial glucose excursions by suppressing glucagon secretion and slowing gastric emptying [30–32]. In long-term (6–12 months) clinical studies of insulin-treated type 1 or type 2 diabetic patients, adjunctive treatment with pramlintide lowered HbA<sub>1c</sub> levels and produced a significant and sustained reduction in body weight, particularly in those patients who were overweight at baseline [33–36].

Based on the anorexigenic effects of amylin in rodents and the weight-lowering effect of pramlintide in patients with diabetes, we hypothesised that pramlintide would enhance satiety and decrease ad libitum food intake in humans. This hypothesis was formally tested in the present randomised, double-blind, placebo-controlled crossover study, which employed a well-established experimental buffet-meal design.

## Subjects and methods

**Subjects** Two groups of subjects were studied: subjects with insulin-treated type 2 diabetes (14 randomised, 11 evaluable) and obese subjects without known diabetes (16 randomised, 15 evaluable). All subjects were male; females were excluded because of the confounding effect of menstrual cycle on hunger and food intake [37].

The primary inclusion criteria required that subjects were between 18 and 70 years of age, were euthyroid (thyroid-stimulating hormone within the normal range), had stable weight (<5% weight change within 2–6 months prior to screening), and had baseline clinical laboratory tests (haematology, clinical chemistry and urinalysis) that were either normal or, if abnormal, judged to be clinically insignificant by the investigator. Subjects also had to be unrestrained eaters, as defined by a score of <11 on Factor 1 (cognitive restraint) of the Three-Factor Eating Questionnaire [38]. The obese non-diabetic subjects had BMIs between 30 and 40 kg/m<sup>2</sup> [39].

Subjects with insulin-treated type 2 diabetes were required to have a BMI between 20 and 40 kg/m<sup>2</sup>, HbA<sub>1c</sub> levels between 6.5 and 10.0%, a stable insulin regimen for at least 6 months prior to screening, and were to be free of severe hypoglycaemia (defined as episodes requiring intravenous glucose or glucagon) for at least 4 weeks prior to screening. Subjects with type 2 diabetes using metformin in conjunction with insulin were required to be on a stable dose of metformin for at least 3 months prior to screening ( $\leq 2,000$  mg/day) and were to be free of clinically relevant gastrointestinal side effects.

Subjects were excluded if they had any of the following: (1) a clinically significant history or presence of active cardiac disease; (2) untreated or poorly controlled hypertension; (3) gastrointestinal, hepatic, renal, autoimmune or haematological disease; (4) malignant disease requiring chemotherapy; (5) eating disorders such as bulimia or anorexia nervosa; or (6) an acute febrile illness within 2 weeks of screening. Subjects were also excluded if they had a history of alcohol or drug abuse, had donated blood within 30 days of screening, had received any investigational drug within 90 days of screening, or were currently being treated with systemic steroids, anti-obesity agents, antidepressants, antipsychotic drugs or drugs known to affect gastrointestinal motility.

The study was designed in accordance with the Declaration of Helsinki and was approved by the Human Ethics Committee of the Royal Adelaide Hospital. All patients provided informed consent prior to randomisation.

**Study design** This was a single-centre, randomised, double-blind, placebo-controlled, two-period crossover study. Subjects were instructed to report to the clinical research unit in the morning following an overnight fast (from 22.00 hours). Each study visit was separated by at least 72 h, and subjects were to maintain routine dietary habits between study visits. On both study days, subjects completed the same standardised meal test.

Prior to the meal test, an intravenous cannula was placed in an antecubital vein for blood sampling ( $t=-60$  min). Venous blood samples were collected over 5.5 h, and assessments using visual analogue scales (VASs) were conducted at time points similar to those used for venous blood sample collection.

At  $t=0$  min, subjects received a single s.c. injection of either 120  $\mu$ g of pramlintide (Amylin Pharmaceuticals, San Diego, CA, USA) or placebo, immediately followed by in-

gestion of a standardised preload meal. A dose of 120  $\mu\text{g}$  was chosen because it has been widely studied in long-term trials [33–35], it has been shown to elicit a sustained reduction in body weight [33–36], and is intended for clinical use in insulin-treated subjects with type 2 diabetes. The preload meal consisted of 125 g of banana blended with 150 ml of low-fat (2%) milk and 150 ml of water (estimated energy content  $\sim$ 189 kcal; 6 g protein, 36 g carbohydrate, 3 g fat), and was consumed within 3 min. At  $t=0$  min, subjects with type 2 diabetes also injected an appropriate dose of their usual mealtime insulin (same dose on both occasions) as determined by the investigator. For those type 2 diabetic subjects using metformin in conjunction with insulin, the usual metformin dose was omitted on the morning of the two study days.

At  $t=60$  min, 1 h later, subjects were offered an ad libitum buffet meal for a period of 45 min ( $t=60$ –105 min). The buffet meal provided food items in quantities exceeding expected intake. Food selections included ham and chicken sandwiches prepared on white or whole-wheat bread with a variety of condiments/toppings (cheese, tomato, lettuce, cucumber and mayonnaise), and yogurt, fruit salad, individual fruits, custard, orange juice and coffee.

**Measurements/endpoints** Total energy and macronutrient intake was calculated using the DIET/4 program (Xyris Software, Highgate Hill, QLD, Australia) [9]. Meal duration (i.e. total duration of eating at the buffet meal) was also recorded.

Subjective ratings of hunger, fullness and nausea over the course of the meal test were repeatedly recorded using a paper-based, standardised 100-mm VAS [8, 40]. Subjects were carefully trained in the use of the VAS prior to each experiment.

Two satiety quotients were calculated as integrated measures of satiety and food intake [41]. The prandial satiety quotient, which is an indicator of suppression of hunger during a meal, normalised for energy intake at the meal, was calculated as follows: [VAS hunger rating obtained prior to the buffet ( $t=1.0$  h) minus AS hunger rating obtained when the subject finished eating] divided by total energy intake at the buffet meal. The postprandial satiety quotient, which is an indicator of the return of hunger after a meal, normalised for energy intake at the meal, was calculated as follows: [VAS hunger rating obtained 5 h after the beginning of the buffet meal ( $t=5.0$  h) minus VAS hunger rating obtained when the subject finished eating] divided by total energy intake at the buffet meal.

Blood samples were repeatedly collected for the measurement of postprandial hormonal and metabolic responses, including plasma concentrations of CCK (RIA; intra- and inter-assay precision is  $\leq 9$  and 27%, respectively) [42, 43], total GLP-1 (RIA; intra- and inter-assay precision is  $\leq 15$  and 18%, respectively), active GLP-1 (ELISA; intra- and inter-assay precision is  $\leq 9$  and 13%, respectively), total peptide YY (PYY) (RIA; both intra- and inter-assay precision is  $\leq 9\%$ ) [44], and triglycerides (enzyme-based assay using a Hitachi 917 Biochemical Analyzer; intra- and inter-assay precision is  $\leq 2$  and 3%, respectively). Triglyceride

assays were run by Dorevitch Laboratory Services (Camberwell, VIC, Australia), whereas total and active GLP-1 and total PYY assays were run by Linco Research (St Charles, MO, USA).

**Statistical analyses** The evaluable population was used for all efficacy analyses, whereas the intent-to-treat (ITT) population (all randomised subjects) was used for the safety analyses. Of 30 randomised subjects (ITT), 26 comprised the evaluable population. Four subjects were excluded from the efficacy analyses: two subjects (one subject with type 2 diabetes and one obese subject) did not consume any food at the buffet meal (thus precluding the assessment of meal duration, satiety quotient, and postprandial hormone and triglyceride responses), one subject with type 2 diabetes did not consume the entire preload meal, and one subject with type 2 diabetes withdrew prematurely due to adverse events.

Total energy and macronutrient intake and meal duration were summarised descriptively according to diabetes status and treatment received. Incremental VAS hunger, fullness and nausea score profiles, and incremental CCK, GLP-1, PYY and triglyceride concentration–time profiles, were calculated by subtracting the baseline score (collected at  $t=0$  min) from the score at each successive time point. The robustness of the rating results for hunger, fullness and nausea was tested by analysing the respective VAS data with and without individual outlying values. Both analyses yielded comparable results, and the data presented here do not include outlying values. Each parameter was summarised descriptively. Incremental areas under the per cent score/concentration–time curves (AUCs) from 0–1 h (pre-meal period), 1–5 h (peri- and post-meal period) and 0–5 h (entire period) were calculated using the linear trapezoidal method. The  $\text{AUC}_{0-1 \text{ h}}$  was not calculated for triglycerides.

Data are presented as means $\pm$ SEM. For all parameters measured, the results of the pramlintide and placebo groups were analysed using mixed-effect models that included treatment, treatment sequence and treatment period as fixed effects, and subject within sequence as a random effect. The least squares (LS) means, the LS mean differences between pramlintide and placebo, and the corresponding SEMs, the 95% confidence intervals and  $p$  values for the LS mean differences were derived from the mixed-effect models.

In addition, a correlation analysis was employed to study the relationship between total energy intake and the concomitant pre- vs post-meal change in VAS hunger ratings. The Pearson's correlation coefficients (with  $p$  values) are presented. A  $p$  value of less than 0.05 was considered statistically significant.

## Results

### Baseline characteristics

The baseline characteristics of the insulin-treated type 2 diabetic subjects and the obese non-diabetic subjects are provided in Table 1. On average, the insulin-treated type 2 diabetic subjects were older and weighed less than the obese

**Table 1** Baseline characteristics of the study population

	Type 2 diabetic subjects ( $n=11$ )	Obese non-diabetic subjects ( $n=15$ )
Age (years)	60.2±8.5	41±21
Race, Caucasian/ other (%)	100/0	87/13
Weight (kg)	93.5±15.6	108.6±22.0
Height (cm)	179.9±5.7	177.0±8.2
BMI ( $\text{kg}/\text{m}^2$ )	28.9±4.8	34.4±4.5
Duration of diabetes (years)	14.3±7.9	NA
HbA <sub>1c</sub> (%)	7.8±0.5	5.5±0.2

Values are means±SD, except for race

non-diabetic subjects. The insulin-treated type 2 diabetic subjects, of whom eight (57%) were concomitantly treated with metformin, had a mean HbA<sub>1c</sub> level of ~8%, and a mean diabetes duration of ~14 years.

### Food intake

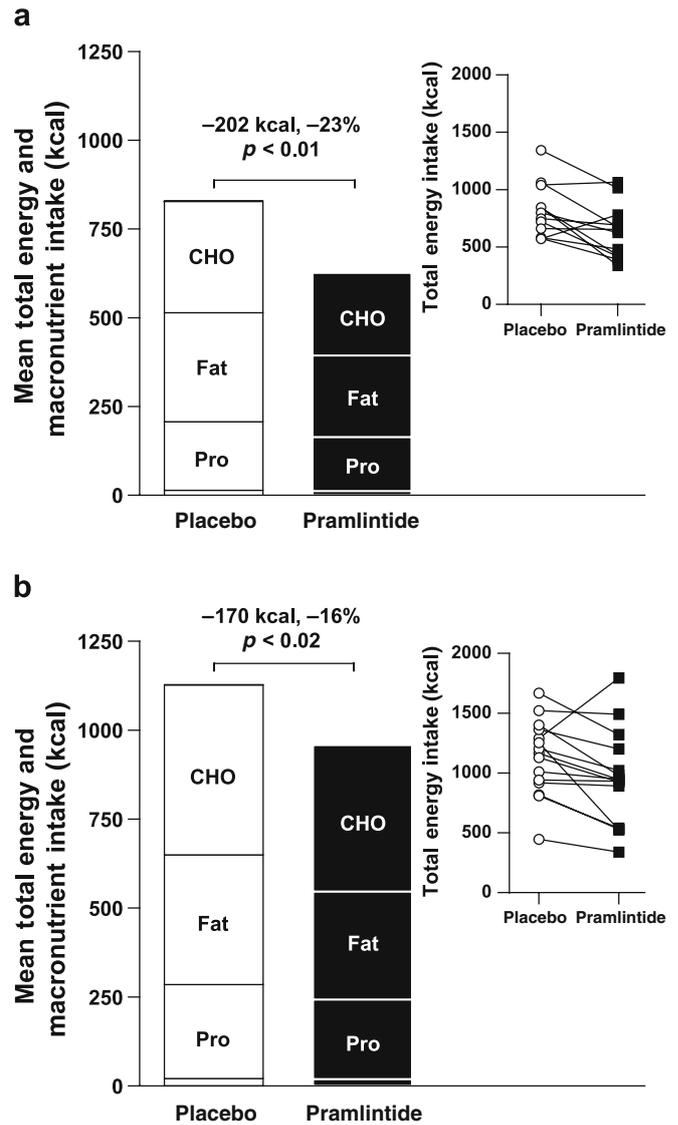
In both groups, the mean total energy intake at the buffet meal was significantly lower following pramlintide than it was following placebo injection (Fig. 1a, b). In the insulin-using type 2 diabetic group, the total energy intake was 829±72 kcal (3,465±301 kJ) following placebo and 627±75 kcal (2,621±314 kJ) following pramlintide injection—a reduction of 202±64 kcal (844±268 kJ) or ~23±8% ( $p<0.01$ ) (Fig. 1a). Of the 11 evaluable subjects with type 2 diabetes, nine (~82%) had lower total energy intake following pramlintide than following placebo injection, while two subjects had greater total energy intake (Fig. 1a inset). In the obese non-diabetic group, the total energy intake was 1,128±81 kcal (4,715±339 kJ) following placebo and 958±100 kcal (4,004±418 kJ) following pramlintide injection—a reduction of 170±68 kcal (711±284 kJ) or ~16±6% ( $p<0.02$ ) (Fig. 1b). Of the 15 obese non-diabetic subjects, 14 (~93%) had lower total energy intake following pramlintide than following placebo injection, while only one subject had a greater total energy intake (Fig. 1b inset).

In both groups, the reduction in mean total energy intake following pramlintide injection was attributable to proportionate reductions in energy derived from fat, carbohydrate and protein, such that the percentage macronutrient composition of the meal was unaffected by treatment (Fig. 1a, b).

The meal duration was similar following placebo and pramlintide injection in both the insulin-treated type 2 diabetic subjects (25±2 vs 25±3 min,  $p=0.80$ ) and the obese non-diabetic subjects (29±2 vs 28±3 min,  $p=0.70$ ).

### VAS ratings of hunger, fullness and nausea

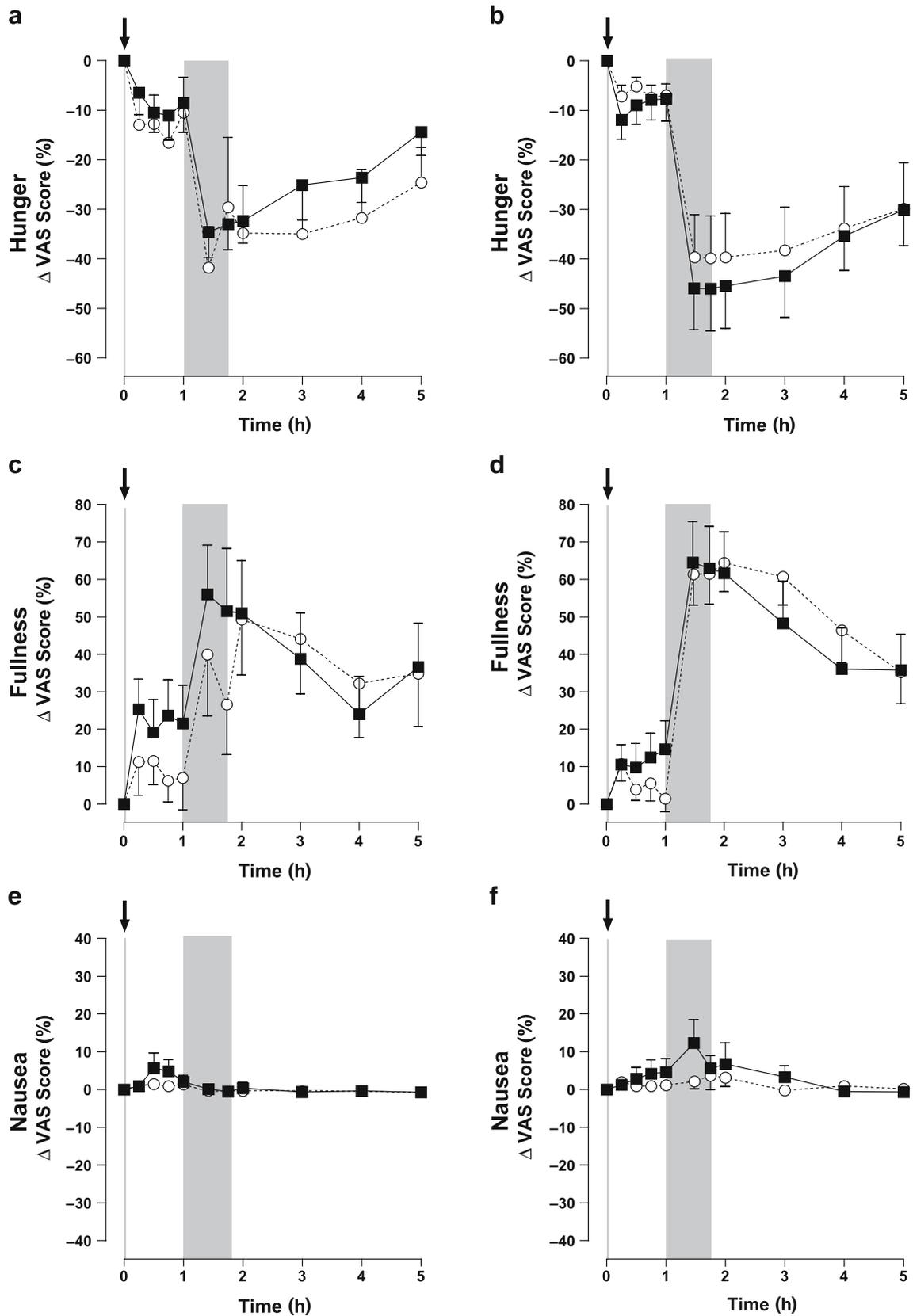
**Hunger** In both groups, and on both occasions, the mean incremental hunger ratings decreased slightly in response to the preload meal (0–1 h), decreased markedly in response to



**Fig. 1** Mean total energy intake (*overall height* of column) and macronutrient intake (*height* of subcolumns) at the ad libitum buffet meal following a single s.c. injection of placebo or 120  $\mu\text{g}$  of pramlintide in 11 subjects with insulin-treated type 2 diabetes (**a**) and 15 obese non-diabetic subjects (**b**). Insets represent individual results for total energy intake. kilocalorie to kilojoule conversion: 1 kcal=4.18 kJ. CHO, carbohydrate; Pro, protein

the ad libitum buffet meal (1–1.75 h), and then gradually reverted towards baseline values during the postprandial period (1.75–5 h) (Fig. 2a, b).

For both groups, the decreases in hunger ratings in response to the preload meal were comparable following placebo and pramlintide injection (Fig. 2a, b). The decreases in hunger ratings in response to the buffet meal, as well as the return of hunger ratings during the postprandial period, were comparable following placebo and pramlintide injection, both among the type 2 diabetic subjects and among the obese non-diabetic subjects, despite the fact that total energy intakes at the buffet meal were 23 and 16% lower, respectively, following pramlintide injection (Fig. 2a, b).



**Fig. 2** Mean ( $\pm$ SEM) incremental changes in VAS ratings of hunger (a, b), fullness (c, d), and nausea (e, f) following a single s.c. injection of placebo (open circles, dotted lines) or 120  $\mu$ g of pramlintide (filled squares, solid lines) in 11 subjects with insulin-treated type 2 diabetes

(a, c, e) and 15 obese non-diabetic subjects (b, d, f) (outliers removed). The arrow/grey line indicates injection of either placebo or pramlintide and liquid pre-load meal; the shaded area indicates the time during which the buffet meal was offered

**Fullness** In both groups, and on both occasions, mean incremental fullness ratings increased slightly in response to the preload meal (0–1 h), increased markedly in response to the buffet meal (1–1.75 h), and then gradually reverted towards baseline values during the postprandial period (1.75–5 h) (Fig. 2c, d).

In both groups, the increases in fullness ratings in response to the preload meal tended to be more marked following pramlintide than following placebo injection, although the differences in the incremental  $AUC_{0-1\text{ h}}$  did not reach statistical significance (Fig. 2c, d).

The increase in fullness ratings in response to the buffet meal, as well as the decline in fullness rating during the postprandial period, were comparable following placebo and pramlintide injection in the type 2 diabetic and obese non-diabetic groups, despite the fact that total energy intake at the buffet meal was 23% and 16% lower respectively, following pramlintide injection (Fig. 2c, d).

**Nausea** Mean nausea ratings only changed minimally throughout the experiments in both groups, with only marginal, non-significant differences found between pramlintide and placebo (Fig. 2e, f).

#### Relationship between total energy intake and hunger suppression during and after the buffet meal: prandial and postprandial satiety quotients

In both groups, there was a positive and significant relationship between total energy intake at the buffet meal, and the concomitant pre- vs post-meal change in VAS hunger ratings (for the type 2 diabetic group  $r=0.50$ ,  $p=0.02$ ; for the obese non-diabetic group  $r=0.43$ ,  $p=0.02$ ) (Fig. 3a). That is, the degree of hunger suppression at the buffet meal increased in proportion to the total amount of energy consumed.

In both groups, the regression line for the relationship between total energy intake and hunger suppression at the buffet meal was shifted downward following pramlintide compared with that calculated following placebo injection (Fig. 3a, b). Consequently, following pramlintide injection, a lower total energy intake was needed to achieve the same degree of hunger suppression, as indicated by the vertical lines in Fig. 3a and b (predicting that an ~200-kcal (~836 kJ) lower total energy intake is needed to achieve 30% hunger suppression).

Consistent with these correlation analyses, the LS mean prandial satiety quotient, an integrated measure of energy intake and hunger suppression during a given meal, was increased following pramlintide compared with that calculated after placebo injection—by 26% in the type 2 diabetic group ( $0.048\pm 0.008$  vs  $0.038\pm 0.008$ ,  $p=0.207$ ) and by 58% in the obese non-diabetic group ( $0.041\pm 0.006$  vs  $0.026\pm 0.006$ ,  $p=0.034$ ) (Fig. 3c, d).

The LS mean postprandial satiety quotient, an integrated measure of energy intake and the return of hunger after a given meal, was also increased following pramlintide compared with that calculated following placebo injection—by 100% in the type 2 diabetic group ( $0.038\pm 0.005$  vs  $0.019$

$\pm 0.006$ ,  $p=0.029$ ) and by 120% in the obese non-diabetic group ( $0.022\pm 0.005$  vs  $0.01\pm 0.005$ ,  $p=0.069$ ) (Fig. 3e, f).

#### Postprandial hormonal analytes and triglyceride excursions

In both groups, following placebo injection, mean incremental plasma concentrations of CCK, total GLP-1, active GLP-1 and total PYY increased slightly in response to the preload meal (0–1 h), increased markedly in response to the buffet meal (1–1.75 h), and then gradually returned towards baseline values during the postprandial period (1.75–5 h) (Fig. 4a–h).

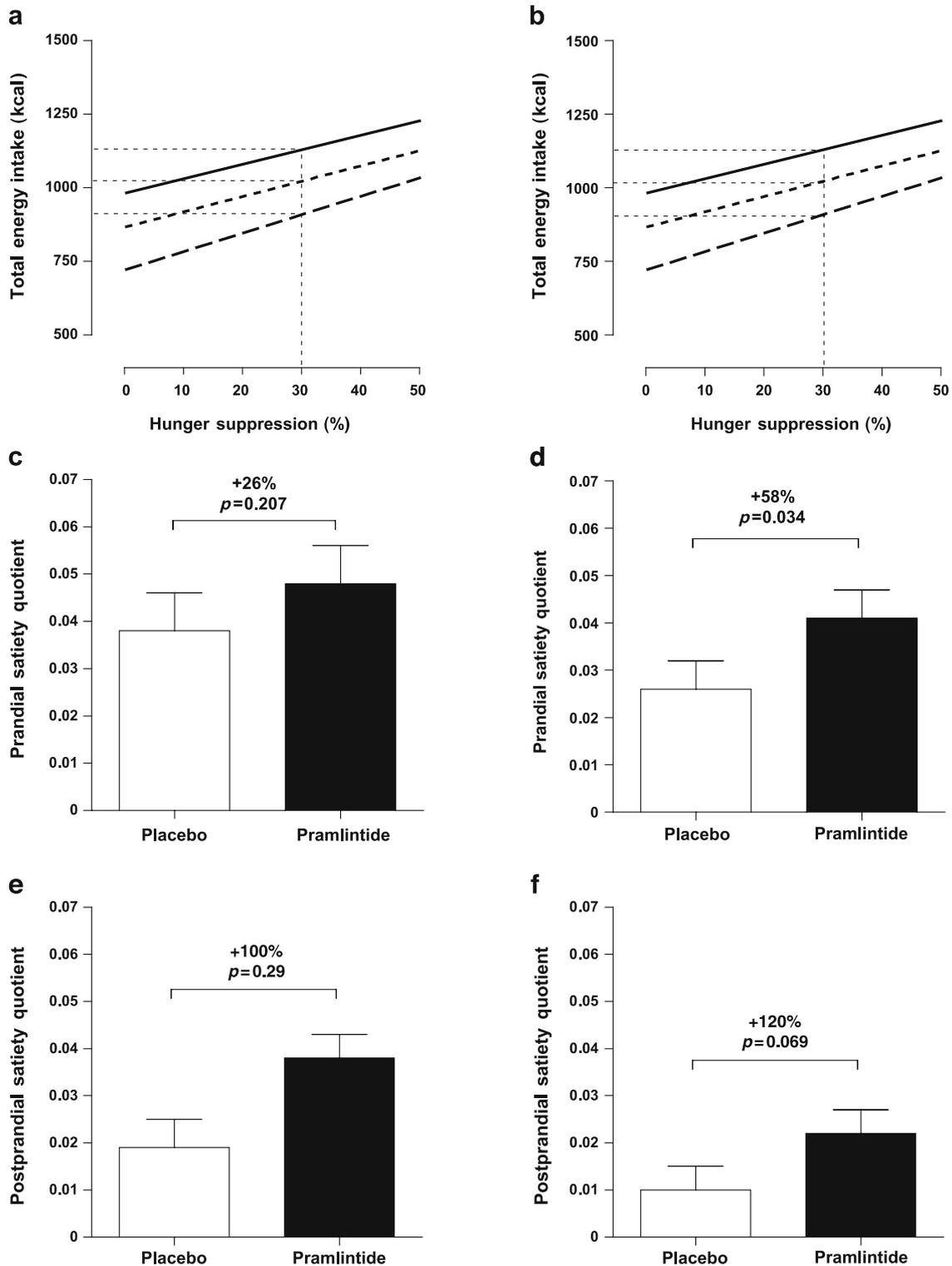
**CCK** In the type 2 diabetic group, the CCK response to the preload meal was attenuated following pramlintide injection compared with that observed following placebo, as indicated by a significant 58% reduction in the incremental  $AUC_{0-1\text{ h}}$  ( $1.3\pm 0.4$  vs  $3.1\pm 0.6$   $\text{pmol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$ ,  $p<0.03$ ). A similar trend was observed in the obese non-diabetic group, although the difference in the  $AUC_{0-1\text{ h}}$  between pramlintide and placebo was not significant ( $2.4\pm 0.7$  vs  $3.0\pm 0.6$   $\text{pmol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$ ,  $p=0.34$ ). In both groups, the CCK response to the buffet meal was not different between pramlintide and placebo (Fig. 4a, b).

**GLP-1 (total and active)** As shown by the >100% reductions in the incremental  $AUC_{0-1\text{ h}}$ , the total GLP-1 response to the preload meal was attenuated following pramlintide injection compared with that observed following placebo in both the type 2 diabetic group ( $-1.8\pm 0.9$  vs  $1.9\pm 0.9$   $\text{pmol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$ ,  $p<0.02$ ) and the obese non-diabetic group ( $-0.3\pm 0.6$  vs  $2.0\pm 0.8$ ,  $p<0.04$ ). In both groups, the active GLP-1 responses to the preload and buffet meals were similar for pramlintide and placebo (Fig. 4e, f).

**PYY (total)** In the type 2 diabetic and obese non-diabetic groups, the total PYY responses to the preload meal tended to be lower following pramlintide injection compared with those observed following placebo injection, although the differences in  $AUC_{0-1\text{ h}}$  were not statistically significant (Fig. 4g, h). The total PYY responses to the buffet meal ( $AUC_{1-5\text{ h}}$ ) were also lower following pramlintide injection in both groups, although the difference in  $AUC_{0-5\text{ h}}$  between pramlintide and placebo only reached statistical significance in the type 2 diabetic group ( $p<0.05$ ).

**Triglycerides** In both groups, following placebo injection, mean incremental serum triglyceride concentrations gradually and progressively increased in response to the pre-load and buffet meals, and were observed to be still rising at the end of the experiment ( $t=5\text{ h}$ ) (Fig. 5a, b).

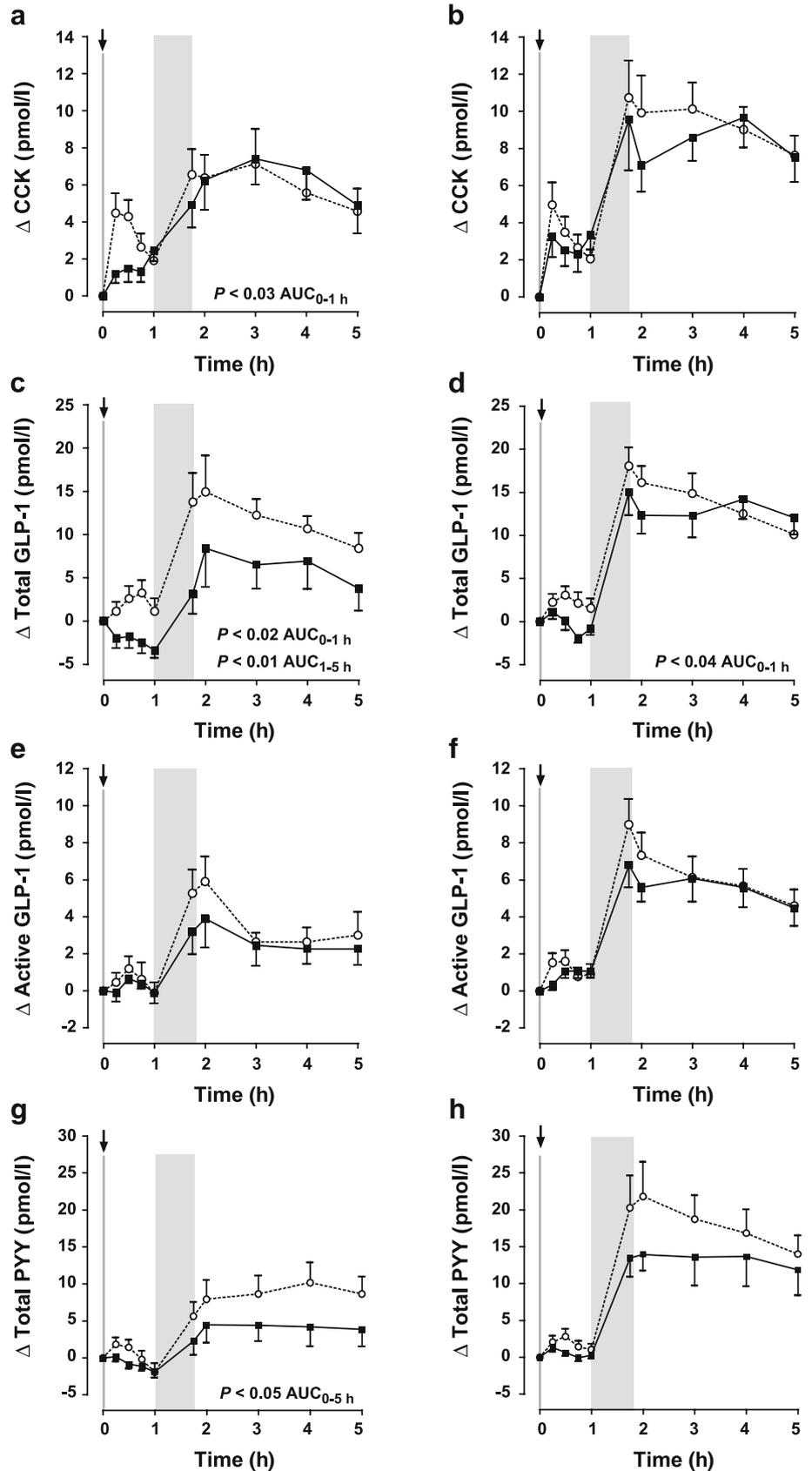
The triglyceride response during the 5 h experiment was attenuated following pramlintide injection compared with that observed following placebo, with reductions in the incremental  $AUC_{0-5\text{ h}}$  of 36% observed in the type 2 diabetic ( $0\pm 0.7$  vs  $3.2\pm 0.8$   $\text{mmol}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$ ,  $p<0.01$ ) and 34% in



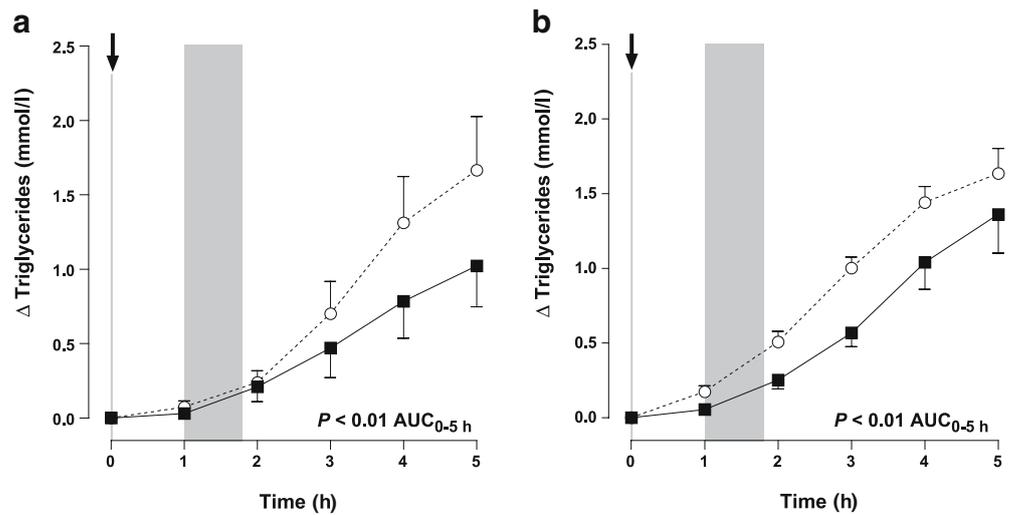
**Fig. 3** Relationship between total energy intake and hunger suppression at the buffet meal: satiety quotients. **a, b** Regression lines for the relationship between total energy intake and hunger suppression (pre- minus post-buffet VAS hunger ratings) (outliers removed). Type 2 diabetes subjects (**a**): placebo-treated (*solid line*)  $y=636.8+6.4x$ , pramlintide-treated (*dashed line*)  $y=400.8+8.4x$ , combined (*dotted line*)  $y=502.8+7.9x$ ;  $r=0.50$ ,  $p=0.02$ . Obese non-diabetic subjects (**b**): placebo-treated (*solid line*)  $y=981.1+4.9x$ , pramlintide-treated (*dashed*

*line*)  $y=720.6+6.3x$ , combined (*dotted line*)  $y=866.4+5.2x$ ;  $r=0.43$ ,  $p=0.02$ . **c–f** LS mean ( $\pm$ SEM) satiety quotients. Prandial satiety quotients (pre- minus post-buffet VAS hunger ratings/total energy intake) (**c, d**) and postprandial satiety quotients (5 h minus individual end of buffet VAS hunger ratings/total energy intake) (**e, f**) following a single s.c. injection of placebo or 120  $\mu$ g of pramlintide in 11 subjects with insulin-treated type 2 diabetes (**c, e**) and 15 obese non-diabetic subjects (**d, f**). kilocalorie to kiloJoule conversion: 1 kcal=4.18 kJ

**Fig. 4** Mean ( $\pm$ SEM) incremental changes in plasma concentrations of CCK (**a, b**), total GLP-1 (**c, d**), active GLP-1 (**e, f**), and total PYY (**g, h**) following a single s.c. injection of placebo (*open circles, dotted lines*) or 120  $\mu$ g of pramlintide (*filled squares, solid lines*) in 11 subjects with insulin-treated type 2 diabetes (**a, c, e, g**) and 15 obese non-diabetic subjects (**b, d, f, h**). The arrow/grey line indicates injection of either placebo or pramlintide and ingestion of the liquid pre-load meal; the shaded area indicates the time during which the buffet meal was offered



**Fig. 5** Mean ( $\pm$ SEM) incremental changes in plasma triglyceride concentrations following a single s.c. injection of placebo (open circles, dotted lines) or 120  $\mu$ g pramlintide (filled squares, solid lines) in 11 subjects with insulin-treated type 2 diabetes (a) and 15 obese non-diabetic subjects (b). The arrow/grey line indicates injection of either placebo or pramlintide and liquid pre-load meal; the shaded area indicates the time during which the buffet meal was offered



the obese non-diabetic ( $2.6 \pm 0.4$  vs  $3.9 \pm 0.3$   $\text{mmol} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$ ,  $p < 0.01$ ) groups.

### Safety

Pramlintide was generally well tolerated, with no serious adverse events occurring. In the type 2 diabetic group, one subject on pramlintide reported mild anorexia and nausea ( $\sim 7$  h after dosing), and one subject on placebo reported reduced appetite. None of the subjects in the obese non-diabetic group reported adverse events whilst on pramlintide, whereas three subjects reported a total of six mild adverse events on placebo (two reports of headache, and one report each of fatigue, diarrhoea, nausea and vomiting).

### Discussion

Several randomised, double-blind, placebo-controlled long-term trials on patients with type 2 diabetes have shown that the addition of pramlintide to pre-existing insulin regimens led to a further improvement in glycaemic control that was consistently accompanied by weight loss [34, 35]. In recent pooled analyses of two long-term type 2 diabetes studies, 26 weeks of treatment with pramlintide (120  $\mu$ g twice daily) led to a significant, placebo-corrected reduction in body weight that was progressive in nature, evident in subjects who did not report nausea, and most pronounced in subjects who were severely obese at baseline (averaging  $\sim 3$  kg in subjects with an entry BMI of  $> 35$   $\text{kg/m}^2$ ) [36].

The results of the present study provide evidence that pramlintide exerts a satiating effect in humans, and that reduced food intake may be a mechanism for the weight loss observed in overweight subjects in long-term clinical diabetes trials with this compound.

Compared with placebo, a single s.c. injection of pramlintide given 1 h prior to an ad libitum buffet meal resulted in a significant reduction in total energy intake that averaged 202 kcal (844 kJ,  $\sim 23\%$ ) in insulin-treated type 2

diabetic subjects and 170 kcal (711 kJ,  $\sim 16\%$ ) in obese non-diabetic subjects. Although differences in study design preclude the direct comparison of our results with those obtained with other peptide hormones, it is noteworthy that the magnitude of the reduction in food intake observed in response to pramlintide is similar to that reported for other gastrointestinal satiety signals, such as GLP-1 [9–11], PYY [13, 14] and CCK [1–5, 7, 8]. In most of the aforementioned studies, peptides were administered via continuous i.v. infusion. In contrast, in the present study, pramlintide was administered as a single preprandial 120- $\mu$ g injection, consistent with the method of administration used in previous long-term trials. Considering that the average daily energy intake of US adults over the past 30 years has increased by an estimated 7% (168 kcal) in males and 22% (335 kcal) in females, as reported in recent NHANES-based CDC estimates [46], and that an  $\sim 20\%$  reduction in ad libitum food intake, if maintained, could translate into a substantial energy deficit over weeks and months, our findings are of considerable clinical relevance.

The reductions in total energy intake induced by pramlintide (compared with placebo) were attributable to proportional reductions in energy derived from fat, carbohydrate and protein, suggesting that pramlintide had no major effect on macronutrient preference. However, this finding should be interpreted with caution, given that most of the food items offered at the ad libitum buffet meal were of mixed macronutrient composition, with relatively few high-fat and high-carbohydrate choices that might have been required to detect a putative drug effect.

The finding that pramlintide did not affect mean meal duration, despite its effect on total energy intake, should also be interpreted with caution. In a rodent study by Lutz et al. [46], peripheral amylin administration reduced meal duration and meal size, leading the authors to suggest that amylin may be an important physiological mediator of meal termination. Given that the plasma concentration of pramlintide peaks at 20–30 min after s.c. injection [28], it is possible that meal duration would be reduced if pramlintide

were injected within 15 min, rather than 1 h, prior to an ad libitum meal.

Analyses of the VAS data reveal interesting insights into the potential mechanisms underlying the observed reduction in food intake with pramlintide. The preload/buffet meal setting used in the present study was highly informative in this regard, allowing us to assess the effect of pramlintide on hunger, fullness and nausea ratings in the context of both standardised (fixed energy) and ad libitum unrestricted energy meals. The VAS ratings during the first hour after injection suggest that enhanced pre-prandial fullness may have been one contributor to the observed reduction in energy intake with pramlintide, a possibility that could be formally tested in a larger study. Over the period during which the buffet meal was available, subjective ratings of hunger decreased and those of fullness increased to a similar extent with pramlintide and placebo, despite the fact that the mean total energy intake was ~20% lower following pramlintide than that following placebo injection. The finding that less food needed to be consumed to elicit comparable hunger suppression and fullness induction during the meal, and a comparable return of hunger and dissipation of fullness after the meal, suggests that pramlintide enhances the satiating effect of meals during both the prandial and postprandial period. This notion is further supported by our correlation analyses, and the finding that the prandial and postprandial satiety quotients [41] were both increased following pramlintide injection. Compared with the fullness and hunger ratings, mean nausea ratings remained essentially unchanged throughout the two study periods. This observation, and the fact that none of the subjects in the type 2 diabetic and obese non-diabetic groups reported nausea as an adverse event within the 5 h study period after the administration of pramlintide, clearly excludes nausea as the primary explanation for reduced food intake.

To examine whether the effect of pramlintide on food intake may, at least in part, be mediated via effects on other humoral mediators of satiety, including gastrointestinal peptide hormones and/or postprandial lipaemia, we measured the postprandial profiles of plasma CCK, GLP-1 (total and active), total PYY and serum triglycerides. The results indicate that none of these factors appear to account for the observed satiating effect of pramlintide. The responses of CCK, total GLP-1 and total PYY to the pre-load meal, and the overall postprandial triglyceride excursions were attenuated rather than augmented following pramlintide administration. The mechanism(s) underlying the attenuation of the CCK, total GLP-1 and total PYY responses to the pre-load meal with pramlintide remains to be elucidated. The reduced responses may reflect, at least in part, slower gastric emptying of the preload meal [18], thereby decreasing small intestinal nutrient stimulation. However, if this were the dominant mechanism, a diminished response to the buffet meal (which was smaller after pramlintide treatment) would be expected, but this was only evident for PYY (and then only in the type 2 diabetic group). A more likely explanation is that pramlintide inhibits vagal efferents that

modulate CCK, GLP-1 and PYY release during the early postprandial period. This concept is supported by the fact that the early postprandial secretion of CCK, GLP-1 and PYY is under vagal control [47], and non-clinical [18] and clinical [32] evidence that the effect of amylin/pramlintide on gastric emptying is also vagally mediated. Of note, in rodents with bilateral vagotomy, the effect of amylin on food intake is preserved, while the effect on gastric emptying is abolished, indicating that the anorexigenic effect of amylin is, at least partly, dissociated from its effect on gastrointestinal motility [48].

Slowed gastric emptying, together with reduced fat intake at the buffet meal, may have contributed, however, to the observed reduction in postprandial triglyceride excursions following pramlintide injection. The latter finding is consistent with previous results in type 1 diabetic patients and is of potential clinical significance given that postprandial hypertriglyceridaemia is increasingly recognised as being an important cardiovascular risk factor [49].

In summary, a single s. c. injection of pramlintide (120 µg) given 1 h prior to a buffet meal significantly reduced ad libitum food intake in subjects with insulin-treated type 2 diabetes and in obese non-diabetic subjects. The VAS results indicate that the reduction in food intake is clearly dissociated from the perception of nausea, and is more likely due to an enhancement of the satiating effect of food. These findings are consistent with the well-characterised anorexigenic effect of amylin in rodents, and suggest that reduced food intake may be a mechanism for the weight loss that has been consistently observed in long-term trials with pramlintide in overweight patients with diabetes.

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